Reference: *Biol. Bull.* **168:** 189–213. (April, 1985)

SELECTED ASPECTS OF LENS DIFFERENTIATION

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ABSTRACT

Recent reviews and papers regarding lens differentiation have been considered in this brief review intended for a general biological readership. Structural and biochemical bases for the initial formation and the continuing growth of the lens are discussed as embryological and maturational processes. Cell division, conversion of epithelial to fiber cells, elongation and invagination, and positional differences in individual lenses are discussed. Emphasis on protein aggregation and chemical changes in fiber cell membranes leads to the conclusion that cataracts not due specifically to toxic, environmental, or genetic factors are merely the result of terminal lens differentiation in which the lens nucleus scatters light excessively.

INTRODUCTION

The ocular lens has often been used as a model in studies of cell differentiation, and many articles and reviews have been published on this subject. It is not the purpose of this review to summarize all of the previous literature, but it refers to recent selected papers. The material presented is that portion of the information with which the author is most familiar. Since lens cells continue to differentiate throughout the life of the individual, the following major differentiation processes are going on throughout life.

Lens epithelial cells grow and divide, and become fiber cells as they invaginate and elongate. Older fiber cells are relocated centrally due to the encapsulated nature of the lens. Post-synthetic modification of the lens proteins (*i.e.*, crystallins) leads to aggregation and to altered fiber cell membranes especially toward the oldest central portion of the lens, a collection of events that eventually leads to light-scattering in the lens nucleus.

Ocular lens biochemistry and structure have been remarkably preserved throughout the vertebrate phylum. The lens usually is of a spherical or spheroid shape, sometimes having a more flattened anterior surface. Teleosts, elasmobranchs, and many rodents have nearly spherical lenses, while those of humans, cows, rabbits, and squirrels are spheroids with anterior surface flattened. Generally, spheroid lenses accomodate passively by being stretched thin (for far vision) or relaxed thick (for near vision). However, the non-elastic spherical fish lenses (*i.e.*, teleost and elasmobranch) must also perform the refractive function of the cornea, since the refractive index of sea water is nearly the same as that of the cornea. Lenses of sharks thus may be moved forward and back to allow for accomodation (Gilbert, 1984).

Received 17 January 1985; accepted 25 January 1985.

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The lens develops very early in embryogenesis from ectodermal anlagen. In most animals, the lens is fully developed at birth, and it grows throughout life, although the rate of growth plateaus in late adulthood. There is a progression of cellular changes that converts the epithelial cells from a monolayer over the anterior aspect of the lens (just beneath the connective tissue capsule) into elongated cortical fiber cells that are relocated internally. Further changes that accompany internalization are a gradual loss both of fiber cell nuclei and other subcellular particles (*i.e.*, mitochondria and ribosomes). Cortical fiber cells are then relocated into the lens interior (*i.e.*, the lens nucleus). As this process continues, much of the cytoplasm is lost, and the fiber cells become a concentrated collection of concentric cell membranes in the nucleus with much aggregated (formerly soluble) protein associated by both covalent and noncovalent bonds.

As a result of the process outlined above, the nuclear region of the lens becomes a depository for the oldest and most differentiated cells. An increase in aggregated proteins and relative losses of cytoplasm and decrease in water content (*i.e.*, dehydration) contribute to the enhancement of light-scattering in the nucleus, and the ability to maintain transparency there is diminished. This is clearly observed by slit-lamp examination of the lens in most species. Figure 1 illustrates such nuclear light-scattering in human and elasmobranch lenses.

Lens differentiation can be considered in two different phases: embryological and maturational. Relatively speaking, embryological lens differentiation takes a very short time to be completed (*i.e.*, several months). This phase of differentiation establishes the tissue as having outer anterior epithelial cells and interior elongated fiber cells. The maturational process continues for the life of the individual (*i.e.*, as long as a century in man), and it merely adds new layers of fiber cells over the old. Figures 2a and b illustrate embryological development both schematically and with a chick eye photomicrograph. Figure 3 schematically illustrates maturational differentiation.

Embryological Differentiation

Embryogenesis is the first stage of lens differentiation. Ectodermal cells become epithelial cells at the lens placode stage. It is well-known that a number of factors stimulate lens induction at this time, but the number and types have not been totally elucidated. Agreement has been reached among researchers (Simonneau *et al.*, 1983) that the neural retina provides growth factors that stimulate further lens development from this point on. Recently, *in vitro* studies have shown that these neural factors, substances in serum, and specific hormonal factors can stimulate lens epithelial cell division in cultures and that differentiation into fiber cells and even lentoid bodies occurs readily (Harding *et al.*, 1971).

The second stage of embryological differentiation is elongation. Neural factors stimulate ectodermal cell division and lead to invagination and elongation of these epithelial cells into primary lens fiber cells of the cortex (Arruti and Courtois, 1978). In embryogenesis, lens ectodermal cells elongate so as to form a vesicle which separates the posterior of the tissue from the retina. Ectodermal cells convert anteriorly into an epithelium under the connective tissue capsule. A solid tissue forms as the vesicle is filled with internalized fiber cells that have increased in number due to epithelial cell division, migration, elongation, and invagination. The process of epithelial cell division at the equatorial or bow region of the lens,

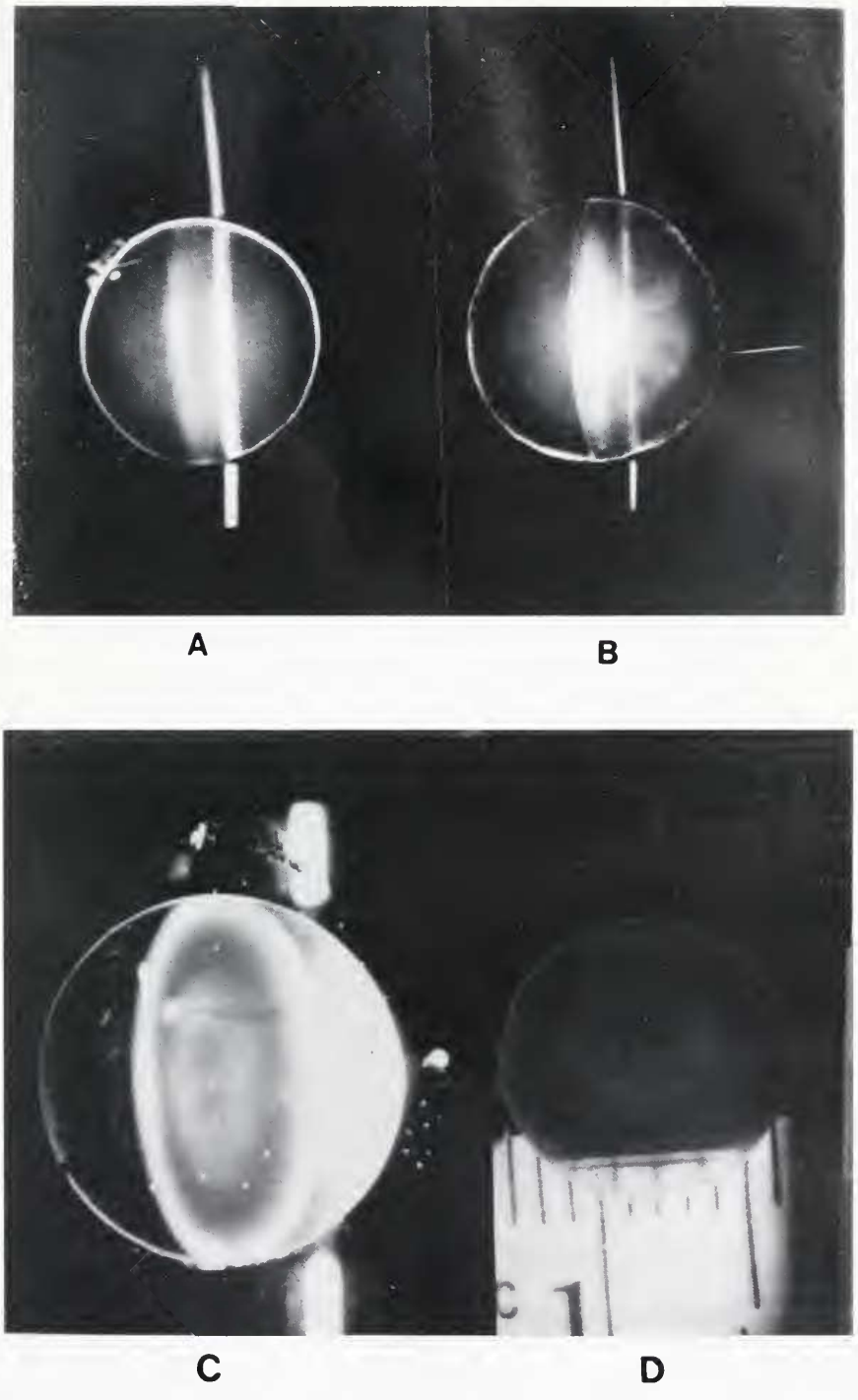

FIGURE 1. Photographs of human and elasmobranch lenses made with slit lamp illumination (A, B, C) so as to exaggerate light-scattering areas. A. Human normal lens, 55-yr-old; B. Nuclear cataractous lens; C. Adult smooth dogfish lens; D. Adult skate lens (anterior view photograph).

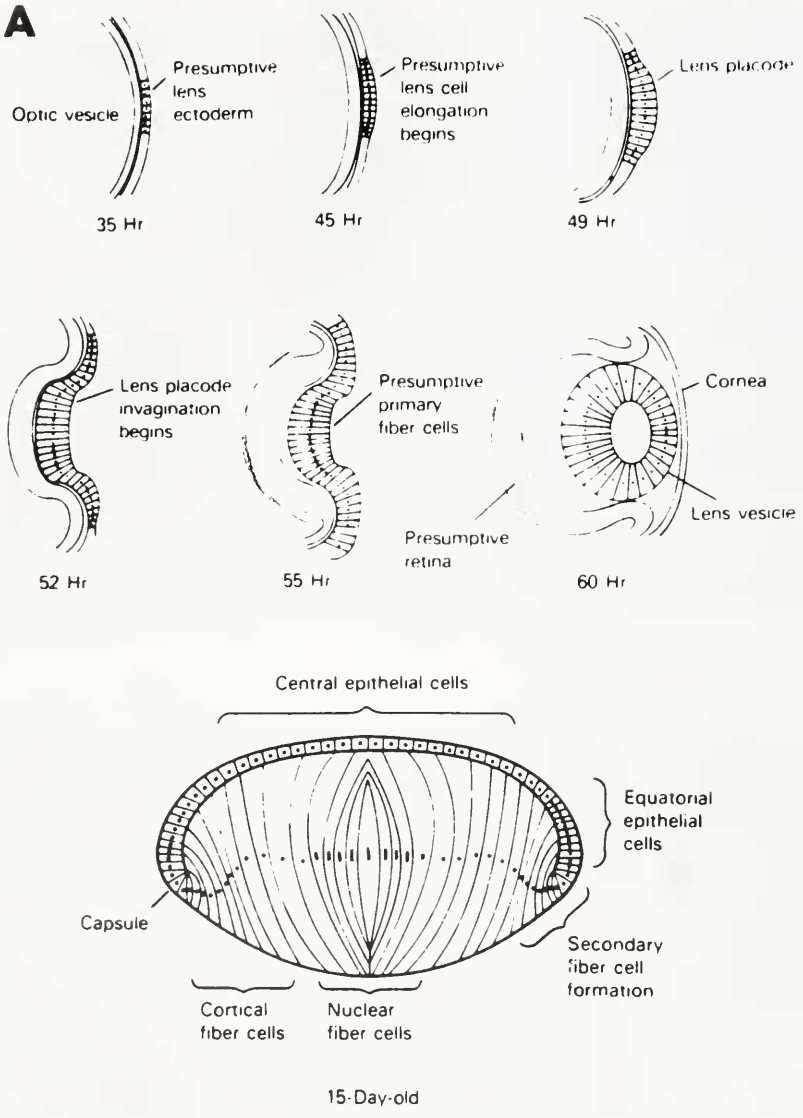

FIGURE 2. A. Embryogenesis of the chick lens from 35 h to 15 days of age. B. Photomicrograph of a cross section of a 4-day-old chick eye that illustrates the differentiation process in the lens (after Piatigorsky, 1981).

invagination, and elongation continues throughout the life of the individual, but at a constantly declining rate.

Chemical factors that are present in neural retina extracts stimulate elongation of lens epithelial cells and crystallin synthesis in rats (Simonneau *et al.*, 1983). Lentropin, a 60,000 dalton heat-labile glycoprotein from the vitreous humor, also stimulates delayed crystallin synthesis in embryonic chick lens epithelium (Piatigorsky, 1981).

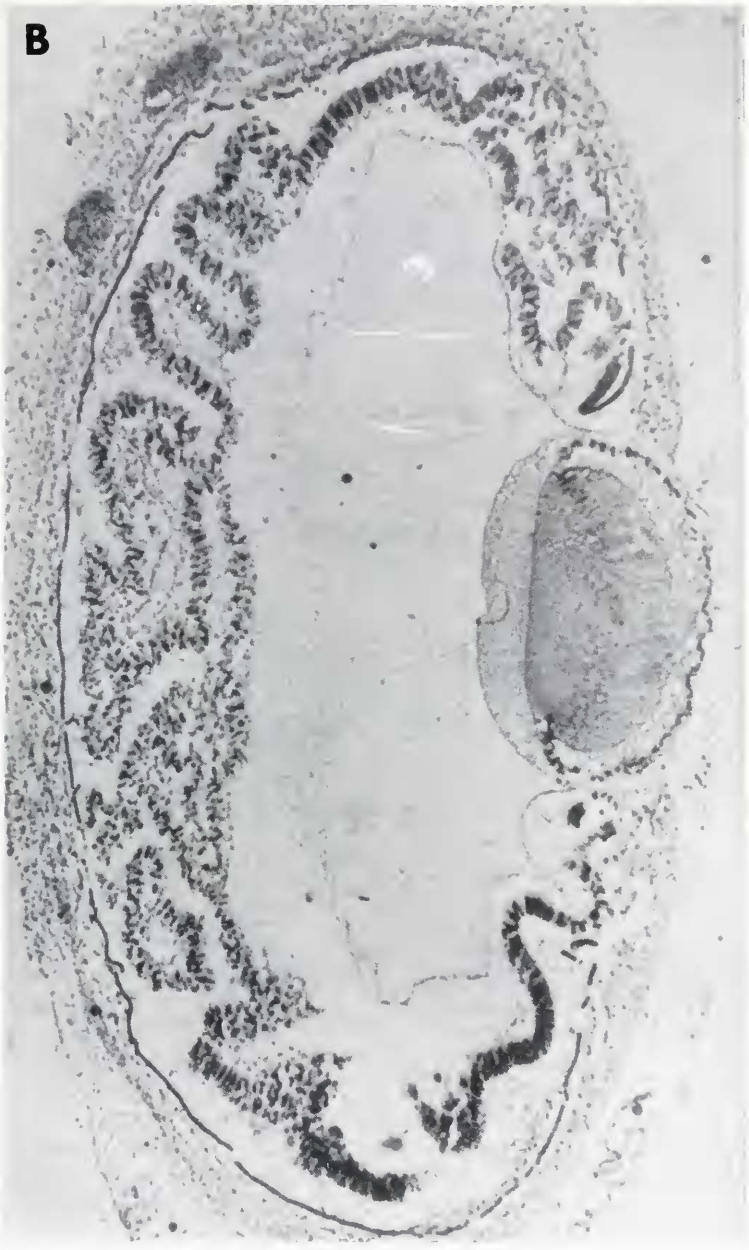

FIGURE 2. (*Continued*)

MOLECULAR EVENTS

Molecular events in differentiation lead finally to the accumulation of crystallins with the distribution found in the mature lens. While lens maturation in adult

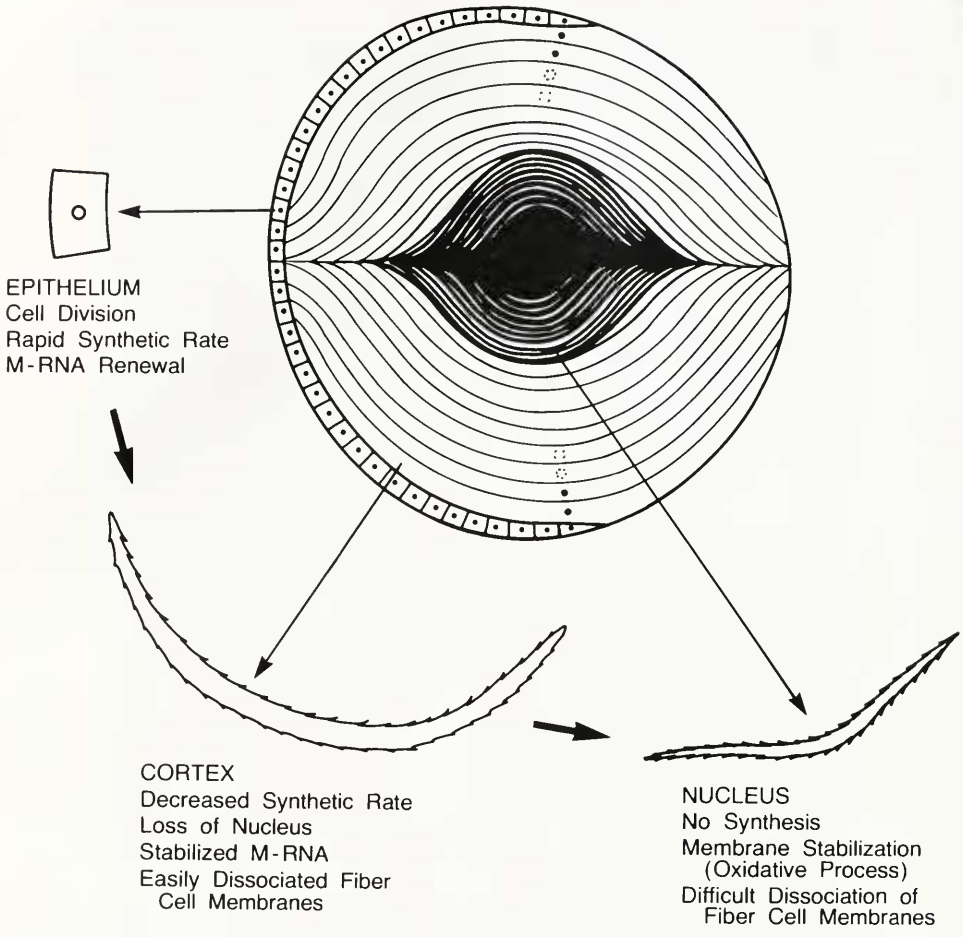

FIGURE 3. Illustration of the maturational differentiation of a schematic lens. Origin of the cells form and growth characteristics of the three major forms of lens cells.

animals results in a distribution of crystallins that will be detailed later in this review, nucleic acid modification also takes place to control this distribution.

Papaconstantinou (1967) has provided a summary of these events that is shown in Figure 4. A controversial concept is that mRNA is stabilized during conversion of epithelial into fiber cells. This conclusion was based upon the use of actinomycin D to prevent mRNA synthesis. However, more recently (Piatigorsky, 1981) has shown that actinomycin D is cytotoxic to adult chicken lens epithelial cells, so that their mRNA instability could be due to toxicity of another sort than mRNA synthesis inhibition. How mRNA stabilization occurs is not known, but hypotheses have been proposed that a ribonuclease inhibitor in the lens is involved (Ortwerth and Byrnes, 1971; Delcour and Pierssens, 1980). Another point is that mRNA state is related to polyribosomal conformation.

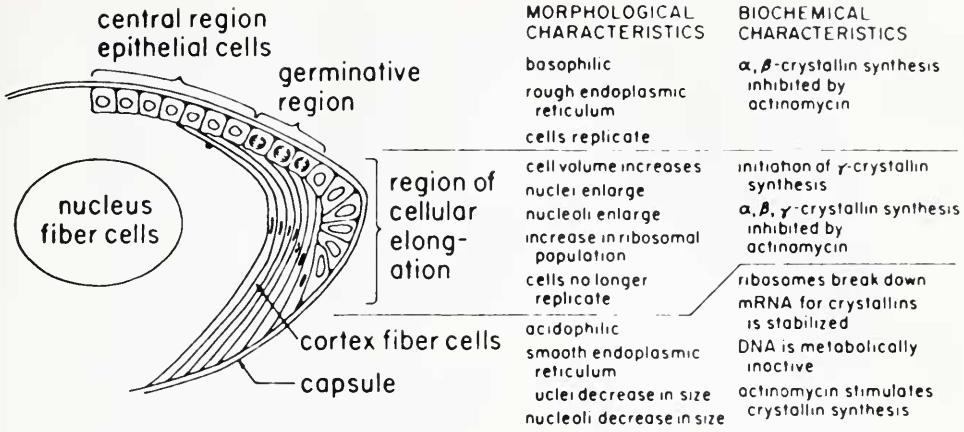

FIGURE 4. Morphology and biochemistry of lens differentiation (after Papaconstantinou, 1967).

One interesting feature of lens differentiation is the loss of cellular organelles and DNA with aging, as defined by position within the lens. As epithelial cells become cortical fiber cells and then nuclear fiber cells, the cell nuclei become pyknotic and the DNA is totally depleted. The cell nucleus first rounds up and then fragments prior to DNA degradation. DNA is thought to become degraded initially by single strand breaks and then is cut into smaller units as determined by alkaline sucrose density gradient studies. Inability to repair DNA may be the manner in which DNA is degraded, but reduction of DNA polymerase does not seem to occur. It is not known how the other organelles are broken down as fiber cells become aged by virtue of their more central positioning in the lens (Modak and Perdue, 1970).

Regions of the lens synthesize RNAs at different rates during the differentiation process. In chick lens, RNA synthesis is most rapid at the equator of the lens where epithelial cells are converted into fiber cells (Modak and Persons, 1971).

As genetic defects are expressed during embryogenesis, they strongly influence the normalcy of the differentiated lens product. These defects eventually lead to disturbances in the transparency of the lens, and results in lenses useless for vision. Many animal models of genetic lens defects based upon specific biochemical lesions have been described. An example is the Nakano mouse model, in which an excess of Na^+/K^+ ATPase inhibitor causes opacity due to osmotic imbalances and swelling (Kinoshita *et al.*, 1974). Another is the galactosemia of infants, in which the enzyme U.D.P.G. galactosyl-transferase is lacking and sugar alcohol accumulation leads to osmotic cataract (Chylack, 1981). Other genetic defects that interfere with lens differentiation have been discovered in chickens and mice (Piatigorsky, 1981).

MATURATIONAL DIFFERENTIATION

Two major biochemical processes are the most important events in the maturational differentiation of the lens. The first is the change in the synthesis and accumulation of the lens crystallins, and the second is protein aggregation either as a separate process or one that involves the association of the protein with the membranes of the fiber cells.

Figures 5 through 8 illustrate some of the well-known features of the three major lens crystallins. Figure 5 demonstrates the sedimentation rate of an aqueous extract of a dogfish lens. Figure 6 shows both the undenatured (shark) and denatured (human) polyacrylamide gel electrophoretic profiles of aqueous buffer homogenate supernatants, and Figures 7 and 8 show the Sephadex G200 resolvable proteins of the bovine lens, and their profiles by electrophoresis. Three similar structural soluble protein classes are found in the lenses of nearly all vertebrates, with the exception of the birds and the reptiles. These have been termed alpha (α), beta (β) and gamma (γ) crystallins, but in birds and reptiles, γ-crystallins are lacking and another protein named delta (δ) crystallin is present. α-crystallin is the largest of the crystallins, with molecular weights ranging from 700,000 to 900,000 daltons, but they are composed of multiples of subunits in the 20,000 to 23,000 dalton range. This protein is composed of two acidic and two basic types of subunits. β-crystallins have heterogeneous sizes ranging from 30,000 to 250,000 daltons. The γ-crystallins are a group of single-chain multi-isomeric proteins with a molecular weight of approximately 20,000 daltons, and they differ greatly in isoelectric points and thus in their electrophoretic mobilities (*i.e.*, α is the most electronegative and γ is the least). Due to a high tryptophan content, γ-crystallins are the most UV-absorbing and fluorescent lens proteins, and they exhibit the greatest molar absorptivity. They are also the most reactive since they contain the greatest -SH content of all the crystallins. Delta (δ) crystallins of birds and reptiles are distinctly (immunologically) different from mammalian crystallins and they are composed of four subunits of approximately 50,000 daltons each, which all differ from each other immunologically, in isoelectric points, and in amino acid compositions (Bloemendal, 1981). When the various crystallins are denatured in SDS, the bands in Figure 7 are observed by polyacrylamide gel electrophoresis.

In the several stages of differentiation, questions arise about the order of appearance and the location in the lens of these crystallins. The distribution of the crystallins varies in different species. For example, α-crystallins appear anteriorly first in rats and mice in the central anterior lens at the stage of ectodermal invagination, while β- and γ-crystallin appearance follows. In newts, β-crystallins appears first, when the primary fiber cells begin to form the lens vesicle; γ- and α-crystallins appear subsequently. However, in the chicken (Delta) crystallin is the first to appear at the lens placode stage, and is followed by the appearance of β- and then α-crystallin. There appears to be a relationship between the formation of the crystallins and the process of cell division, in that while α-crystallin is synthesized in all metabolically active lens cell, β- and γ-crystallins are not readily synthesized in lens cells that are actively dividing (Piatigorsky, 1981). But even though the lens epithelium contains dividing cells, small amounts of β- and γ-crystallins are still found to be present (McAvoy, 1981).

Generally speaking, there is a species difference in the relative levels of the three crystallins. While in bovine and human lenses α-crystallins are present at the highest levels, amphibians and elamobranch lenses contain mainly γ-crystallin. In terms of distribution within the lens, it appears that α-crystallins predominate in the epithelium and outer cortex whereas γ-crystallins predominate in the inner nucleus (McAvoy, 1981).

Lens Membranes

Due to lens growth processes and the conservative nature of the fiber cells, the nucleus grows larger with increasing age, as shown in the dogfish (Fig. 9). Changes

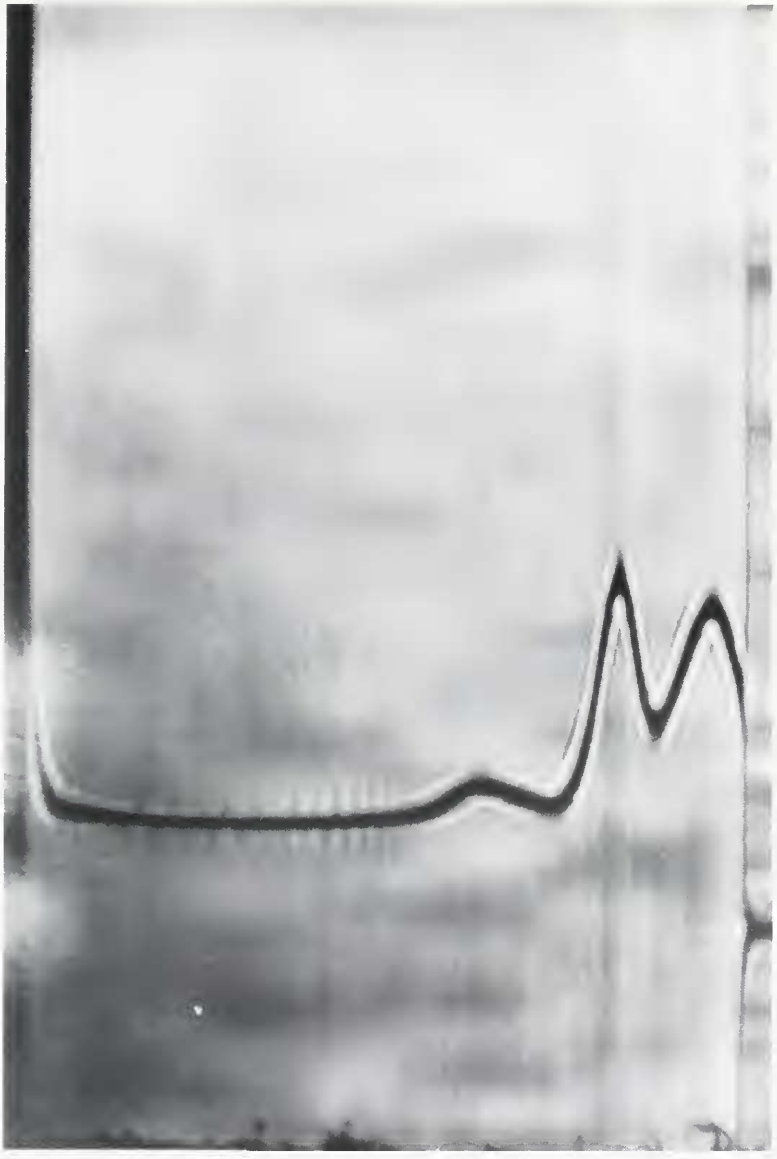

FIGURE 5. Analytical ultracentrifugation (left to right) of the total water-soluble extract of a dogfish lens illustrating the three lens crystallins α- (the heaviest and furthest migrating), β- (intermediate in molecular weight), and γ- (the lightest and slowest migrating). Note also the fringes (to the left) representing high molecular weight colloidal protein aggregates.

in the thickness and in the interdigitating knob structures of the fiber cells of the skate are shown in Figure 10.

Lens nuclei contain some of the longest-lived cell membranes in biological systems. Two views of the structure and extrinsic protein binding to lens fiber cell

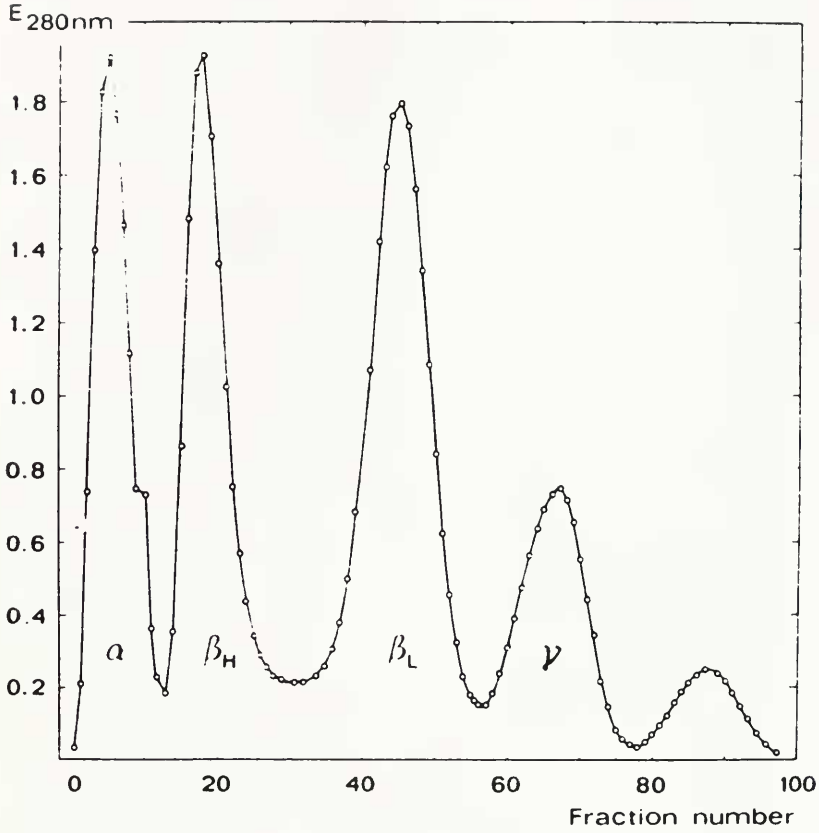

FIGURE 6. Separation of the bovine lens crystallins using Sephadex G 200 (after Bloemendal, 1981). H = heavy, L = light.

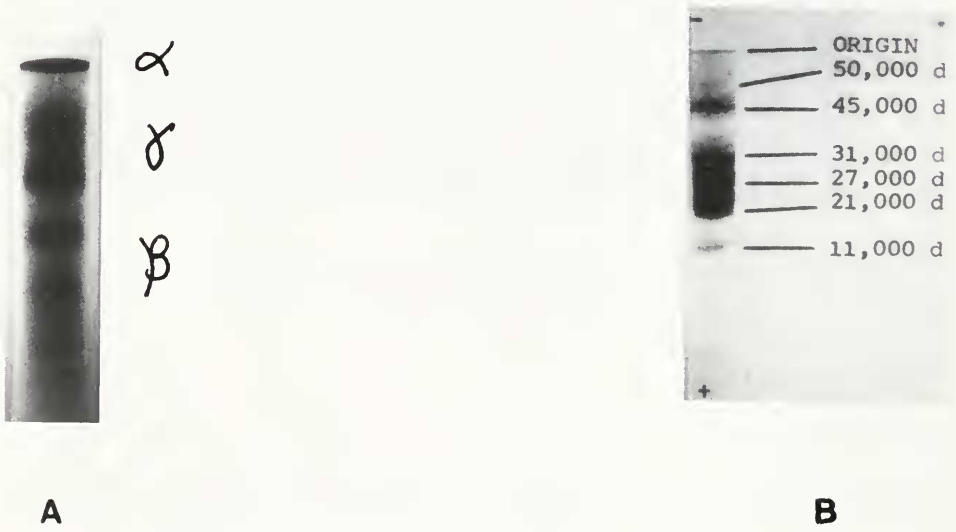

FIGURE 7. Polyacrylamide gel electrophoresis of lens water-soluble extracts. A. Tris glycine buffer solvent; shark lens extract. B. SDS-phosphate buffer solvent; human lens extract.

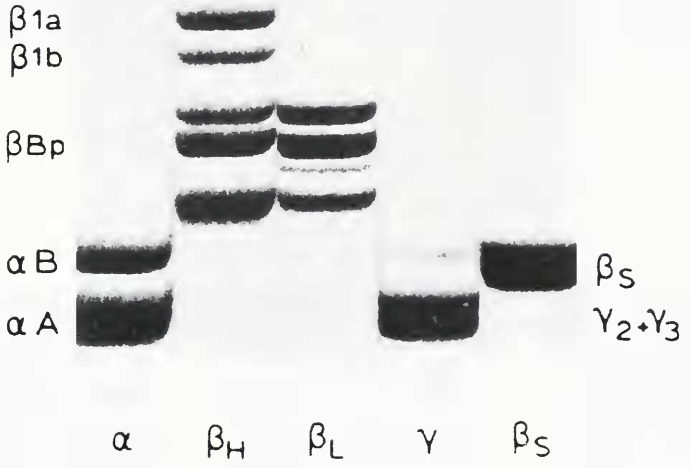

FIGURE 8. SDS polyacrylamide gel electrophoresis of Sephadex G 200 separated bovine lens crystallin subunits (after Bloemendal, 1981). B_5 is a low molecular weight beta (27,000 daltons) that resembles gamma components 2 and 3.

membranes are illustrated in Figure 11 (Spector *et al.*, 1979; Broekhuyse, 1981). Not only do the cytoplasmic elements differentiate as lens epithelial cells become fiber cells, but the cell membranes also change substantially in this way. It is clear that with the loss of cytoplasm and organelles as fiber cells are relocated toward the lens core, the fiber cell membranes collect much bound non-membrane proteins. This increases the protein-to-lipid ratio so as to produce an abnormal membrane environment. There is also a dehydration in the nuclear core as compared with the outer portions of the lens.

Another important influence on fiber cell membranes relative to differentiation is the accumulation of formerly water-soluble proteins in an aggregated form and an unfolding of these proteins so as to expose reactive side groups and to stimulate unnatural protein:protein interactions. The exposure of SH groups is an example of this process, and it has been shown by many investigators (Roy and Spector, 1976; Bloemendal, 1981). Spector *et al.* (1979) have shown that a protein of 23,000 dalton molecular weight has a special affinity for membrane surface proteins in human

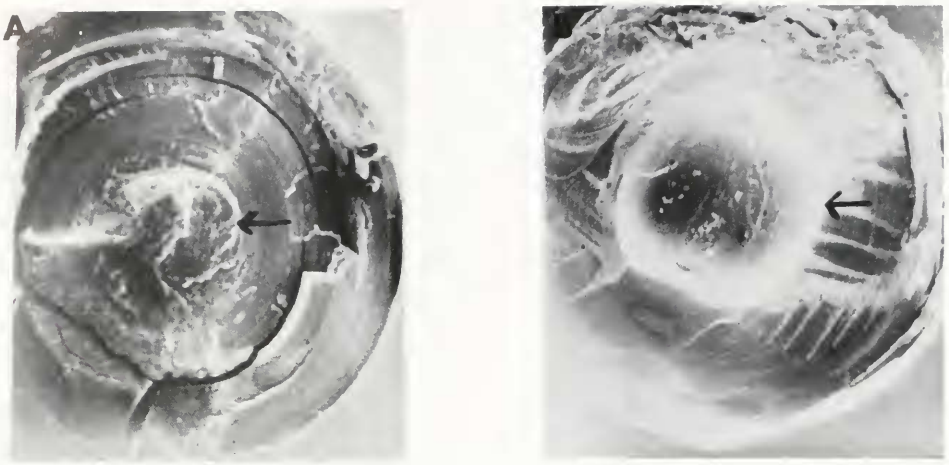

FIGURE 9. Growth of the lens nucleus in the dogfish as shown by scanning electron microscopy of an animal (A) 10 inches in length; and (B) the lens of an animal 45 inches in length. The arrows represent the outer limits of the nuclear region of each lens. Reduction: A, 1 cm = 0.09 cm; B, 1 cm = 0.25 cm.

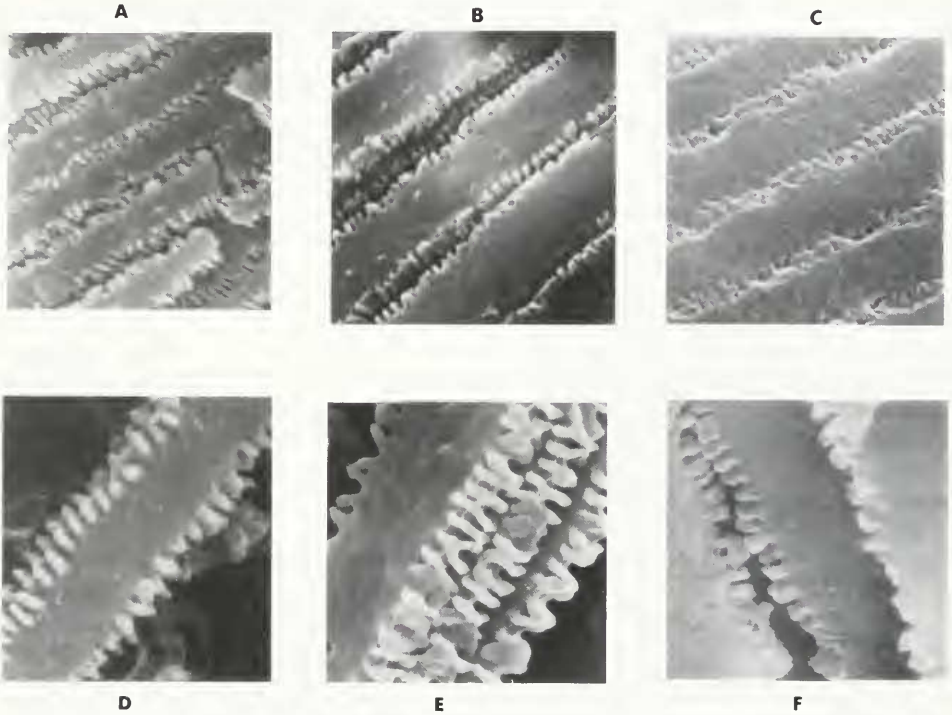

FIGURE 10. Scanning electron microscopic view of the alterations in the morphology of dogfish lens fiber cells relative to age and position in the lens. A, B, C at 2000×; D, E, F at 5000×. A, D. Nuclear fiber cells of a 15-inch-long animal; B, E. Cortical fiber cells of 36-inch-long animal; C, F. Nuclear fiber cells of the 36-inch-long animal.

cataracts via -SH to -SS reactions, and that other lens crystallins such as γ-crystallin also react with 23,000 dalton proteins. In this way, areas of large scale aggregation result along the inner fiber cell membranes. Other forms of covalent bonding between formerly soluble proteins and membrane proteins occur. Figure 11 illustrates the above (Horwitz, unpub. data).

Another aspect of lens fiber membrane protein changes with differentiation involves the intrinsic membrane protein of 26 to 27,000 daltons that represents the major protein of the gap junction (see Fig. 12). With aging, maturation, and position in the lens, the relative amount of the main intrinsic protein (MIP) to the other membrane proteins increases (Horwitz *et al.*, 1979; Zigman *et al.*, 1982).

Extraction of fiber cells consecutively with aqueous buffer, 8 *M* urea, 1% SDS, and then 1% SDS plus 50 m*M* DTT indicates the stability of the membrane to dissolution (see Fig. 13 for the dogfish lens). Table I indicates further how separated skate lens nuclei and cortices respond to such consecutive extractions. It is surprising that SDS alone still leaves much fiber membrane undissolved. DTT added to the

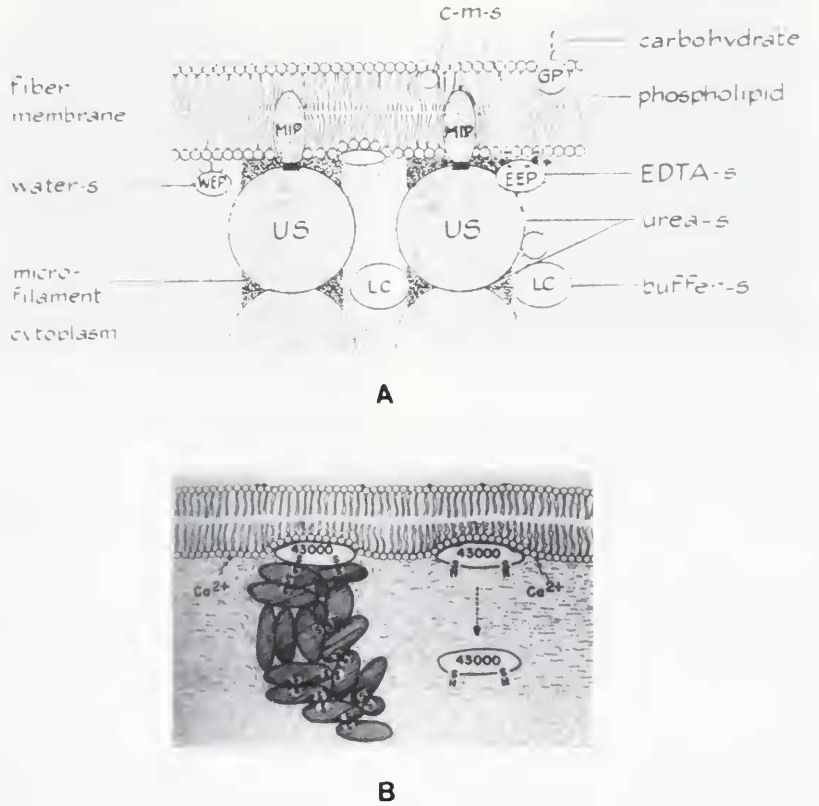

FIGURE 11. Schematics of lens fiber cell membranes. A. Association of water-insoluble but urea-soluble proteins with the fiber cell membrane, and summary of other associated molecular species (after Broekhuyse, 1981). B. Association of the 43,000 dalton water-soluble protein with the lens fiber cell membrane, and aggregation of other soluble crystallins to the membrane via -SS bonding (after Spector *et al.*, 1979).

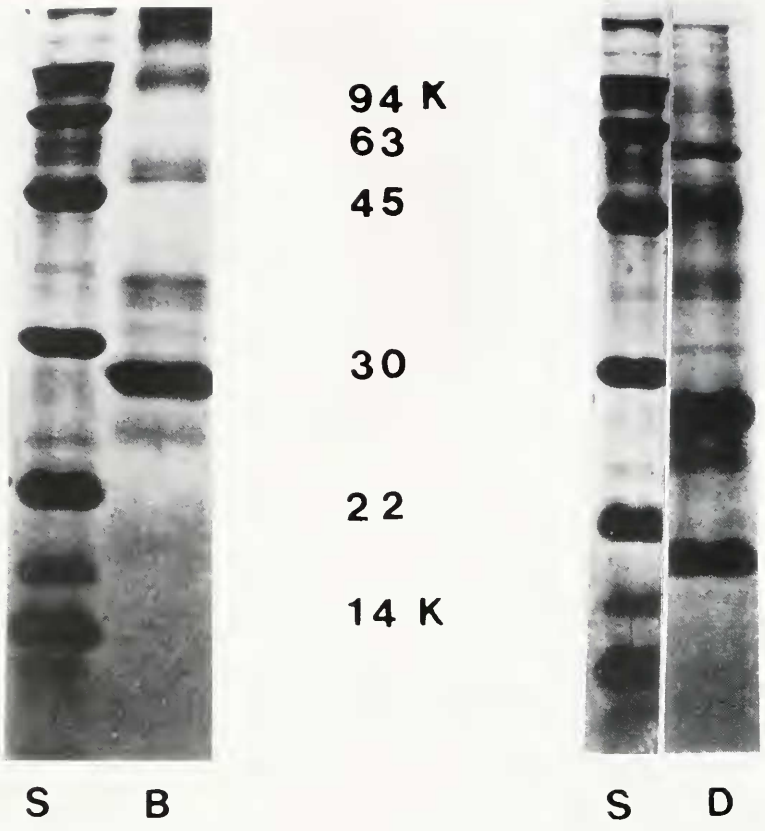

FIGURE 12. SDS-polyacrylamide gel electrophoresis of the urea-insoluble membrane fraction of bovine (B) and dogfish (D) lens homogenates. Note the presence of the 26,000 to 27,000 dalton major bands in both lenses, but other bands that differ in protein and concentration.

SDS apparently does not totally disperse the membrane components (see Zigman *et al.*, 1982).

The major gross change in the nucleus of the lens that represents terminal differentiation is the enhanced light-scattering that is observed in a variety of animals, including elasmobranchs and humans. Such nuclear scattering is the end result of the aggregation due to membrane and soluble protein interactions that occur naturally with aging as a result of lens differentiation. One can thus equate the formation of nuclear opacity (or cataract) to maturation and aging changes that lead to this extreme of the differentiation process (Table I).

CYTOSKELETAL ELEMENTS

An interesting feature of the ocular lens relates to its cytoskeleton and the architecture of the fiber cells. These features change with altered fiber cell shape as they are internalized to the lens nucleus. Figure 14 summarizes the process by illustrating the cytoskeletal elements in the fiber cells. In the cortex the intermediate filaments appear distinct and clearly defined with good connections to the plasma membranes, whereas, the filaments are absent in the nuclear fiber cells and there is clumping of the protein chains to the plasma membranes (see Maisel *et al.*,

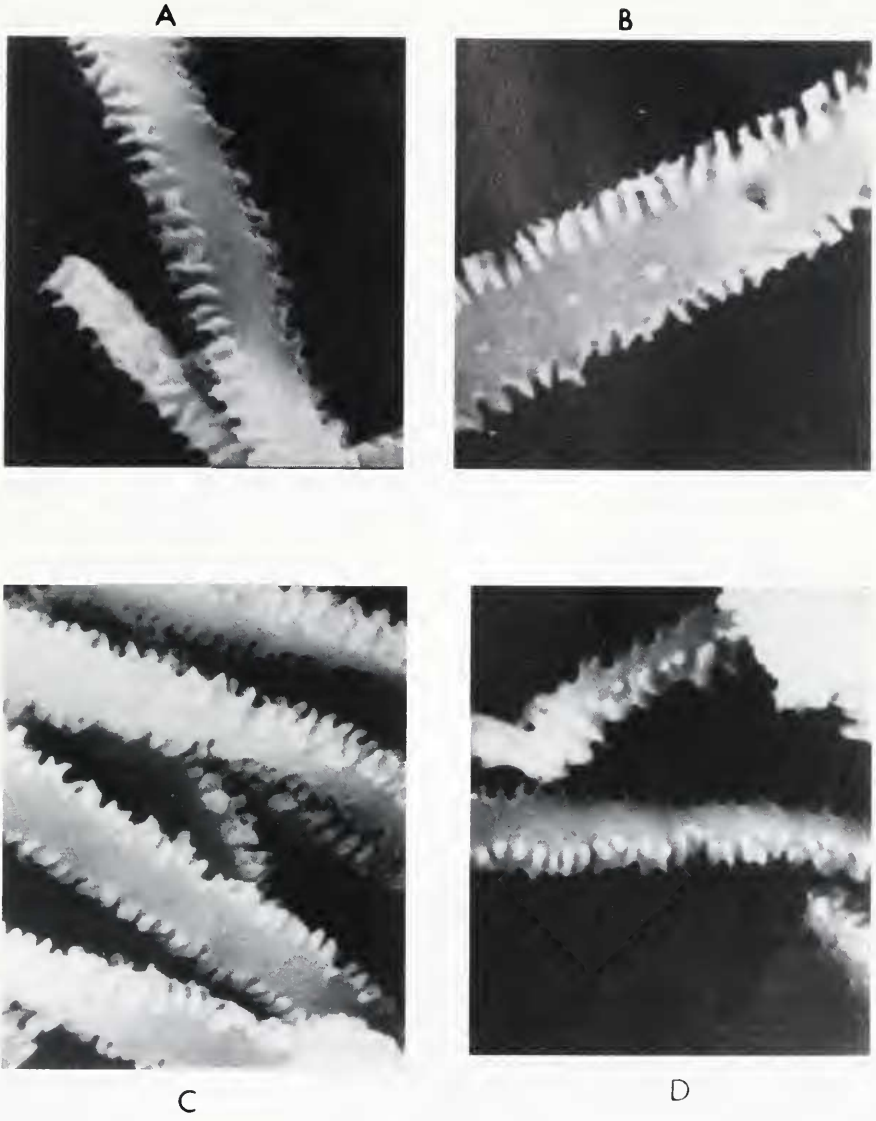

FIGURE 13. Stability of dogfish lens nuclear fiber cells after extraction with tris buffer (A), 8 *M* urea (B), 1% SDS (C), and 1% SDS plus 50 m*M* DTT. (D) 1% SDS plus 50 m*M* DTT for 24 h. The fibers are thinner and appear to disintegrate with time. Only SDS plus DTT was capable of totally disintegrating the fiber membranes.

1981). These structural alterations parallel the diminished elasticity of the nuclear fiber cells.

On a chemical basis, one finds that there are two major elements of the cytoskeleton:vimentin and actin. An SDS polyacrylamide gel of the lens fiber membrane-cytoskeletal complex reveals peptides at 55,000 and at 45,000 daltons, which represent vimentin (55,000) and actin (45,000). Figure 15 illustrates the SDS-polyacrylamide gel profile of skeletal and other membrane elements of cow lenses (Benedetti *et al.*, 1981). The loss of intermediate filaments in the nucleus is

TABLE I

Distribution of insoluble proteins in the skate lens†

a) Total insoluble protein as percent of total protein

Young	Cortex		Nucleus	
(Average* lens weight of 22 mg)	20%		28%	
Adult	Outer cortex	Inner cortex	Outer nucleus	Inner nucleus
(Average* lens weight of 98 mg)	38%	34%	23%	50%

b) Solubility of the insoluble proteins

Young	Cortex		Nucleus	
1) Extracted with 8 *M* urea and then 1% SDS	67%		49%	
2) Extracted with 1% SDS plus 50 m*M* DTT	33%		51%	
Adult	Outer cortex	Inner cortex	Outer nucleus	Inner nucleus
1) Extracted with 8 *M* urea and then 1% SDS	66%	66%	65%	55%
2) Extracted with 1% SDS plus 50 m*M* DTT	34%	35%	35%	45%

* Average of 3 determinations.
† *Raja eglanteria.*

accompanied by the loss of vimentin in the nucleus. Thus, with maturation as judged by position within the lens, the basic protein chemistry and structural elements of the cytoskeleton show great changes. Loss of nuclear zone elasticity with maturation may be the result of these changes.

POSITIONAL MATURATION OF LENS PROTEINS

Lens differentiation continues to take place throughout the lifetime of an individual. Post-translational changes occur in the distribution and interaction of the lens crystallins, as well as in their chemical features. When adult lenses of many species were separated into concentric layers, the soluble crystallin distribution varied similarly in all. While heavier proteins predominated in the total water-soluble fraction of the outer layers, the lower molecular weight species were more abundant toward the lens center or nucleus. In absolute terms as well, the content of the heavier crystallins was greatly diminished in the nuclear core (see Figs. 16–19).

In parallel with the losses of heavier crystallins with internalization, there is an increase in the water-insoluble aggregated fractions. This is true for the water-insoluble protein that can be solubilized in 8 *M* urea and in 1% SDS. But in the water insoluble but SDS plus DTT soluble fraction (*i.e.*, the fiber cell membranes themselves), the insoluble protein remains nearly constant in all layers. The latter fractions would represent the intrinsic lens fiber cell membrane proteins.

It is thought by many lens researchers that the lower molecular weight gamma crystallins have the greatest potential for aggregation and covalent attachment to the fiber cell membranes. This is due to their abundant cysteine and tryptophan

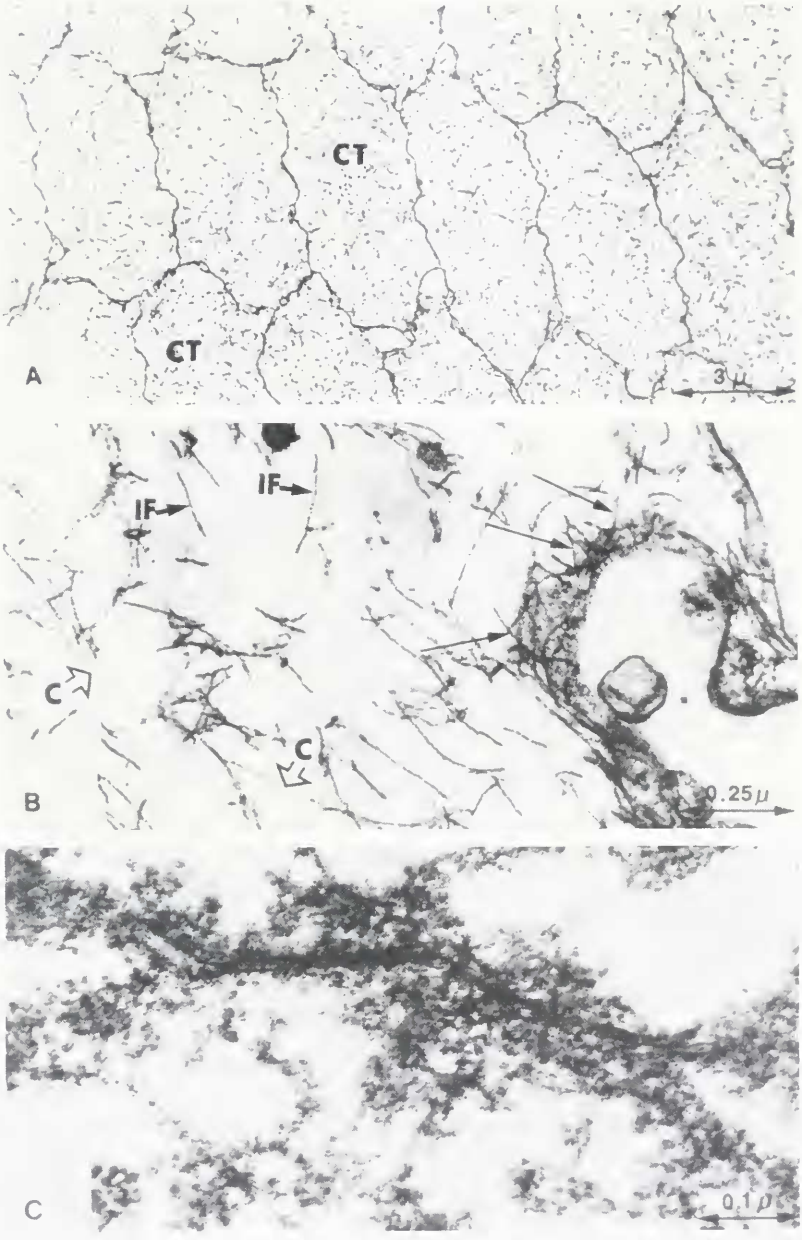

FIGURE 14. Transmission electron microscopic morphology of glycerol extracted fiber cells of the cow lens, prepared so as to illustrate the cytoskeletal elements. A. Presence of cytoskeletal elements in fiber cells cross sections to show relationship between membranes and cytoskeleton; B. cortical intermediate filaments; and C. lack of intermediate filaments in the nuclear preparation and clumping onto the cell membrane (after Maisel *et al.*, 1981).

contents relative to the other crystallins. Both of these amino acids have easily oxidizable side-chains that enhance the reactivity of crystallins due to oxidation reactions. Free -SH groups of γ-crystallins are known to become oxidized, resulting

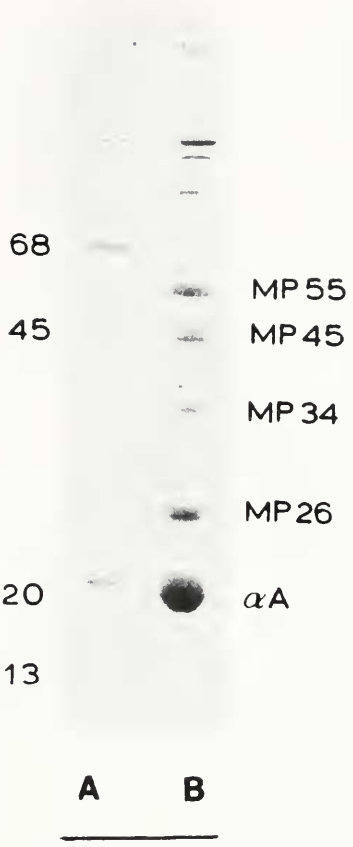

FIGURE 15. SDS polyacrylamide gel electrophoretic profiles of the membrane-associate water-insoluble proteins of the cow lens, showing vimentin (MP 55) and actin (MP 45) of the cytoskeleton, the membrane intrinsic proteins (MP 26, 34), and the A chain of alpha crystallin. A = standards; B = membrane-cytoskeleton extract (after Benedetti *et al.*, 1981).

in the formation of intra- and inter-molecular crosslinks via -SS bond formation (Takemoto and Azari, 1977). Again, due to the conservative growth process of the lens, these post-translational changes in protein side-chains eventually lead to aggregates and light-scattering in the nucleus.

The indole rings of protein tryptophan can also be oxidized to form kynurenine and other products (*i.e.*, beta carbolines, anthranilic acid) that can serve as fluorescent crosslinks between protein molecules (Dillon, 1976). N-formylkynurenine has been isolated from the lens and identified by Pirie (1971) and 3-OH-kynurenine glucoside has been identified in the lens by Van Heyningen (1973) and Bando (1983). These tryptophan products are known photosensitizers. Such photosensitization reactions lead to enhanced protein photo-oxidation that is ultimately found in the nuclear fiber cells. The presence of these aggregated molecules in the nucleus of the lens due to metabolic and radiant energy-induced oxidation has been well-documented, and is most likely responsible for much of the high degree of nuclear light-scattering.

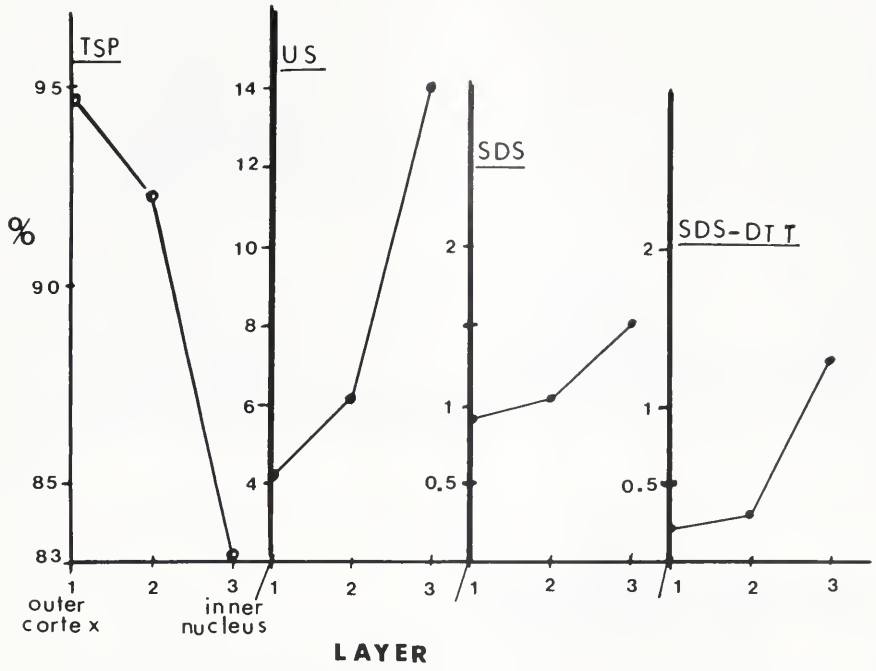

FIGURE 16. Changes in both water-soluble and water-insoluble protein content in concentric layers of the squirrel lens. TSP = water-insoluble; US = urea-soluble; SDS = sodium dodecyl sulphate-soluble; SDS + DTT = SDS plus dithiothreitol soluble proteins.

Positional differences are seen not only with regard to structural proteins, but they also apply to many enzymes of great importance to cell function (see Hockwin and Ohrloff, 1981). For example, the ATPase activity is greatest in the outer layers of the lens, and tend to diminish toward the nucleus (see Table II). With regard to the Na^+/K^+ enzyme, diminished activity in the nucleus leads to light-scattering stimulation due to disturbances in the salt and water balance in this region.

Positional differences between outer less mature and inner more mature regions of the lens apply to several other features of the lens. In one case, there are stable free radicals in the lens whose concentration diminishes toward the nucleus (Fig. 20). It has been hypothesized that the chemical entities that represnt the free radicals are bound to proteins and in fact serve as cross-linking agents. Due to the conservative nature of the lens, such cross-linking agents react with proteins that are finally found in the nucleus, and therefore become quenched with regard to free radical properties (Zigman, 1981).

Another lens component that changes in properties and levels from the outer to inner portions of the lens is the yellow pigment that is present nearly exclusively in diurnally active animals, such as squirrels, monkeys, and humans. The pigments that are present in the lens at birth both in humans and in squirrels, are water-soluble, low molecular weight entities (see Fig. 21). Tentatively speaking, the human pigment is 3-OH kynurenine-glucoside (Bando, 1983; Van Heyningen, 1973), while the squirrel pigment is thought to be n-acetyl,3-OH kynurenine (Van Heyningen, 1973). Both of these are metabolic products of tryptophan via a tryptophan

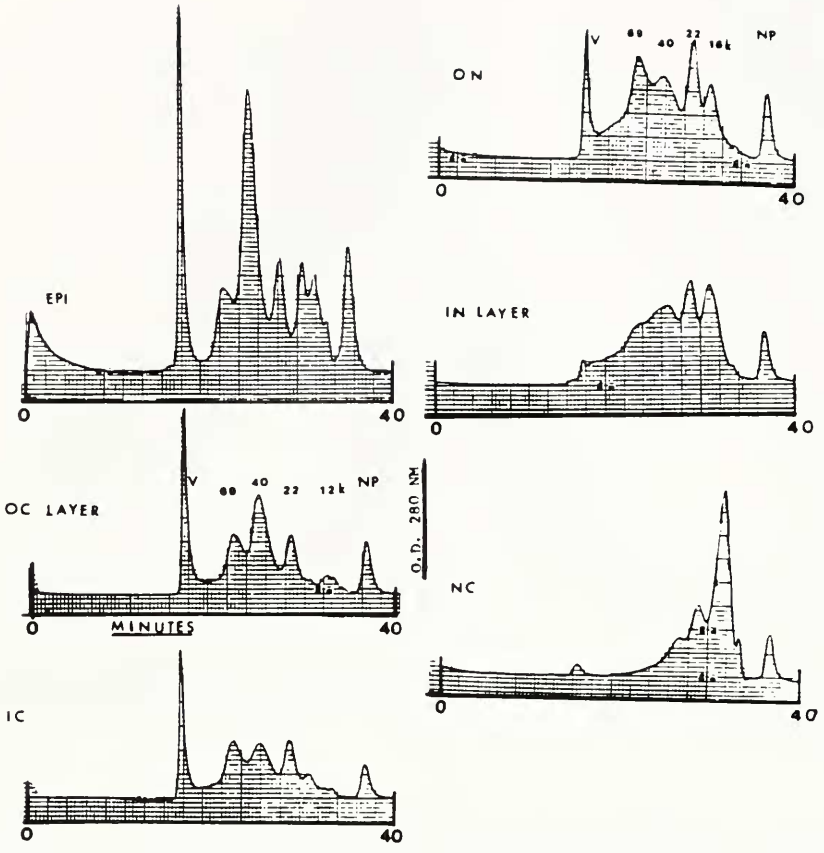

FIGURE 17. High performance liquid chromatography of the water-soluble proteins in the epithelium and five concentric layers of the lens of a squirrel. Note loss of heavy and increase in lighter proteins going from the outer to the inner portion of the lens.

oxygenase enzyme (Van Heyningen, 1973). While in the human, this type of low molecular weight water-soluble lens pigment diminishes with maturation and aging, it appears to increase in concentration as the squirrel matures. In the squirrel lens, the balance of oxidation-reduction of this pigment appears to be maintained with maturation, while in the human lens there is a buildup of new tryptophan oxidation

TABLE II

Distribution of ATPase activity in bovine lens

ATPase of:	Total ATPase	Mg^{++}-ATPase	$Na^{+}K^{+}$-ATPase
Bovine	Micromoles per mg per hour		
Lens epithelium	0.15	0.08	0.07
Lens cortex	0.03	0.02	0.01
Lens nucleus	0.005	0.004	0.001

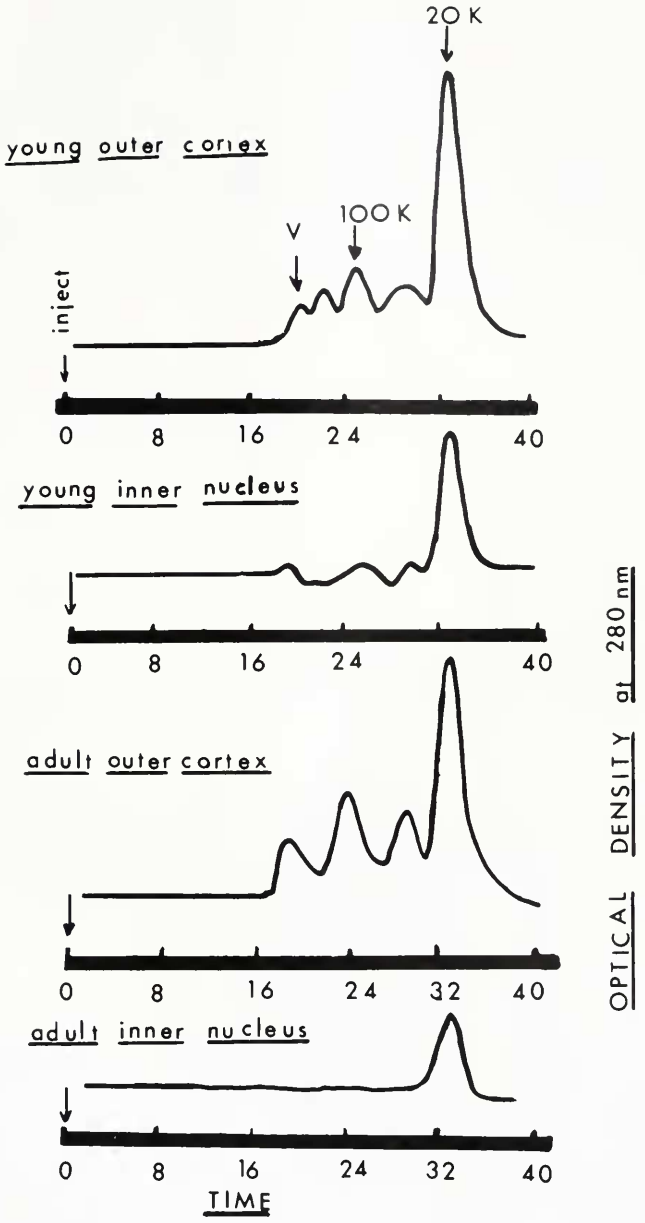

FIGURE 18. HPLC profiles of the outer and inner cortices and nuclei of young (15 inches long) and adult (33 inches long) skates. Note loss of heavy and increase of light protein bands going toward the central core of the lens.

products with aging (*i.e.*, over 30 years of age). For example n-formylkynurenine and B-carbolines become covalently associated with the structural proteins, and via fluorescent cross-links, they enhance aggregation and light-scattering (Dillon *et al.*, 1976). This process of enhanced concentration of protein-bound pigment has only been observed in human lens thus far, perhaps due to the relative longevity of the

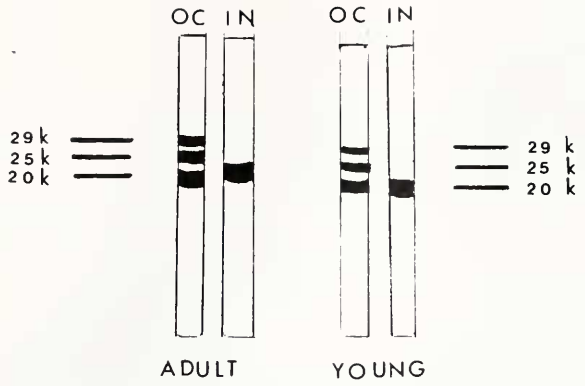

FIGURE 19. SDS-polyacrylamide gel electrophoresis of skate lens proteins. OC = outer cortex; IN = inner nucleus.

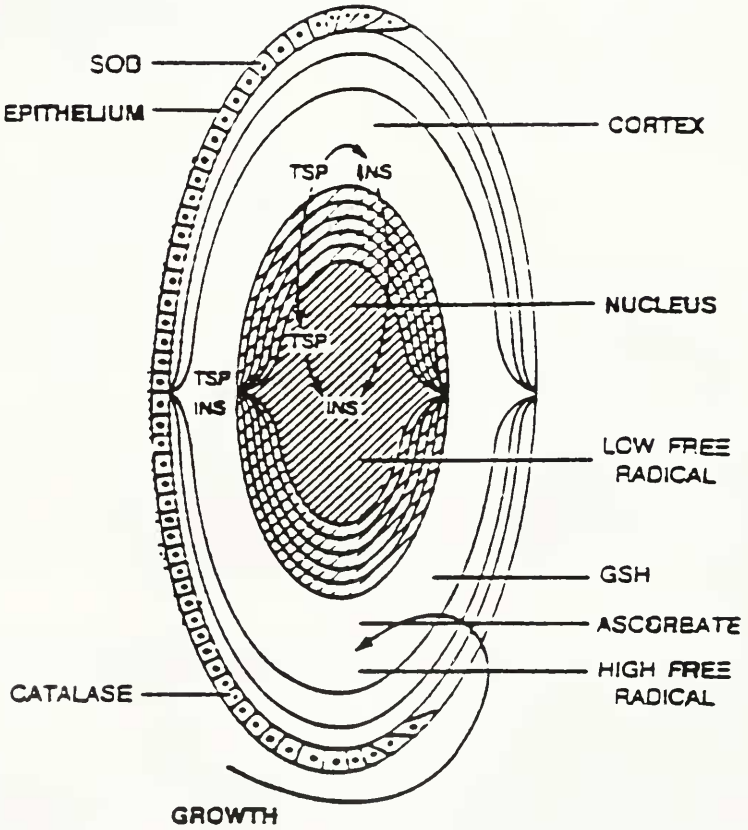

FIGURE 20. Scheme of distribution of stable free radicals, reducing agents, and anti-oxidant enzymes in the ocular lens.

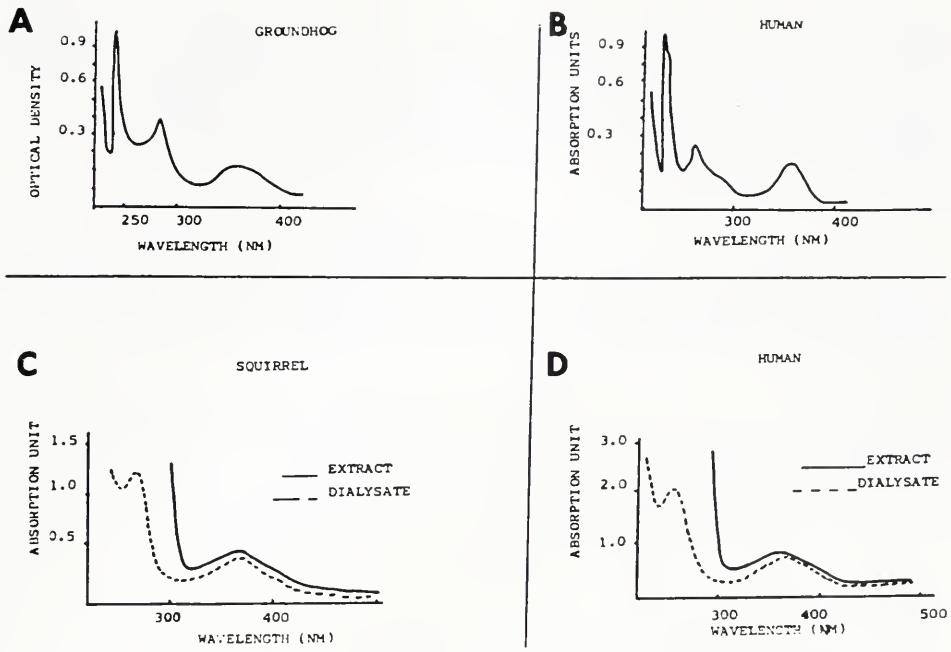

FIGURE 21. Spectral properties of the near-UV and blue-visible absorbing pigments of groundhog, human, and squirrel lenses. A, B are pressure dialyzed non-protein extracts; C, D show both dialyzed and non-dialyzed extracts.

human species. The pigment density becomes quite high only in the nucleus and not in the cortex with an occasional exception. This phenomenon has been ascribed to a paucity of reducing agents (*i.e.*, glutathione, ascorbic acid, etc.) in the nucleus to counteract metabolic oxidant buildup and photosensitized radiant energy absorption effects.

CONCLUSION

This brief review supports the concept that cataract (*i.e.*, excessive extinction of light) is actually a case of terminal differentiation of the lens as influenced by genetic, nutritional, internal biochemical, and environmental factors. With regard to the biochemical influence on cataract formation, the major contributing processes appear to be protein aggregation and association of protein species with the fiber cell membranes leading to light-scattering in the nucleus of the lens. These processes are stimulated by oxidation reactions, and oxidants formed as the result of metabolism play a role in the loss of transparency. Little is yet known about the nutritional aspects of cataract, except that trace elements that function as antioxidants and enzyme cofactors must be maintained to prevent cataract formation. Thus far the genetic influences on cataract formation seem to be mainly related to the lack of enzyme activities which maintain the exchange of substances between the lens and the aqueous humor. Radiant energy seems to be the most influential in cataract formation, in that it has been shown experimentally that free radical formation, fluorescent cross-link stimulation in proteins, and enhanced pigment darkening are stimulated by excessive exposure to the long wavelength light present in the sunlight.

While some of these factors apply more or less to the non-human species, naturally occurring cataract is relatively rare in them. However, the problem of cataract is most acute in humans, in whom all of these factors apply. Longevity is an additional factor that does not apply to most other species. Details of the current studies on human cataract can be found in several recent books (Bloemendal, 1981; Duncan, 1981; Maisel, 1985).

ACKNOWLEDGMENTS

Many thanks to my Laboratory Staff and especially to Bunnie Zigman for their support and hard work. Financial support from Research to Prevent Blindness, Inc., and The National Eye Institute is gratefully acknowledged.

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